

Uptake of Sea Water into the Fluid Spaces of the Prosobranch Gastropod, *Acmaea scutum*¹

BY

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INTRODUCTION

THE CIRCULATORY SYSTEM of most gastropod mollusks functions in part as a hydrostatic skeleton. In the past it was believed that gastropods could incorporate sea water into the blood space to facilitate the expansion of the foot. However, the current view is that, with one exception (the family Naticidae) a constant blood volume in the circulatory system is sufficient to cause the expansion of the foot of gastropods (MORRIS, 1950; CHAPMAN, 1958; BROWN & TURNER, 1962; BROWN, 1964; BERNARD, 1968; RUSSELL-HUNTER & APLEY, 1969). For the Naticidae MORRIS, *op. cit.*, BERNARD, *op. cit.*, and RUSSELL-HUNTER & APLEY, *op. cit.*, have described a system of aquiferous ducts that are separate from the blood system and fill with sea water during foot expansion.

This study shows that the limpet *Acmaea scutum* RATHKE, 1833, which does not use the hydrostatic skeleton to expand the foot in the manner of most gastropods, does however, demonstrate large changes in total volume. Evidence is presented showing that this change in volume results in part from the incorporation of large quantities of sea water directly into the blood space.

MATERIAL AND METHODS

Most limpets used were collected from Jordon River, British Columbia. Laboratory maintenance is described in WEBBER & DEHNEL (1968). *Acmaea scutum* used in methylene blue experiments were collected at Stillwater Cove, Monterey, California.

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Measurements of Volume Change

Animals were maintained in sea water for 24 hours before use. The method of volume measurements was as follows. The animal was taken from the experimental salinity and the foot was compressed gently with absorbent tissue. Volume was determined by removing the limpet from sea water, compressing the foot gently with absorbent tissue, and weighing the volume of sea water displaced by the animal. The method was accurate to ± 0.1 ml. To measure changes in volume, limpets were returned to sea water with the dorsal surface of the shell against substrate so the animal could not attach with the foot. Animals were maintained in this position for the desired time period. The volume of the animal was again determined after gently shaking to remove water from the nuchal cavity and space between the foot and shell. The increase in volume was determined by subtraction. The volume of the soft body parts alone was determined by estimating the volume of the shell separately and subtracting this value from the total volume.

Blood Amaranth Samples

Samples for the determination of blood amaranth values were taken from the visceral sinus through an incision in the foot muscle. Approximately 100 μ liter samples were measured colorimetrically without dilution at 520 m μ , using sea water as a blank.

Blood Inulin Samples

Blood samples for the measurement of inulin were taken from an incision through the foot into the visceral sinus. Fifty microliter aliquots of blood were assayed for inulin by the anthrone method of YOUNG & RAISZ (1952).

RESULTS

When *Acmaea scutum* is removed from the substrate and placed in sea water upside down so it cannot right itself,

over a period of time the soft parts of the limpet appear to swell. The results recorded below document and characterize this change in size.

In measuring the volume increase, it was important that limpets at the start of an experiment had the same relative water content. Before measuring the starting volume the animal was blotted with absorbent tissue to remove excess water. A test using regression slopes (Table 1) showed that all limpets had the same relative water content when the starting volume was measured.

Table 1

Regression equations of initial water content plotted against dry weight for *Acmaea scutum* used in experiments on uptake of water at constant salinity. The p value indicates the probability level at which the regression slope is significant

average water content	regression equation	P
	$Y = 1.36 + 5.32X$	0.01
83.7% (n = 60)	$Y = 0.81 + 4.32X$	0.01
	$Y = 0.68 + 4.50X$	0.01

The change in volume with time is given in Table 2. The volume of the animals increased even though the salinity of the water was constant. By 6-12 hours in this inverted position, limpets increased the volume of the soft parts by approximately 100%. The variation indicated that only large differences could be considered significant. The purpose of the experiment was to demonstrate that *Acmaea scutum* could show an increase in volume of the soft body parts when held at a constant salinity.

Nature of the Volume Change

The blood and urine of *Acmaea scutum* are essentially sea water. The blood and urine concentrations of the major ions (Na^+ , Cl^- , Mg^{++} , and Ca^{++}) are the same as the corresponding concentrations in sea water (WEBBER & DEHNEL, 1968). Ion values for blood and urine samples taken at all stages during increase in volume remained constant, i. e., were the same as sea water. The observed increase in volume, then, must have been achieved by taking in both ions and water.

Uptake of Amaranth

To determine if the water uptake response involved all fluid spaces of the limpet, the red dye amaranth was

Table 2

Increase in volume of soft body parts of *Acmaea scutum* in 100% sea water. Part A is the number of animals of a sample of 10 showing an increase in soft body parts of 5%. Part B is the mean increase in volume (per cent) for those animals showing an increase of 5%. Part C is the blood concentration of amaranth (mg/l) at a concentration of 0.025 mg/l

Part A

number of animals out of 10 showing increase in volume

Time in hours					
1½	3	6	12	24	48
7	9	10	10	10	10

Part B

means of increase in volume (per cent)

Time in hours					
1½	3	6	12	24	48
70.7	71.7	86.2	98.2	100.3	100.5

Part C

Blood concentration of amaranth

Time in hours						
	1½	3	6	12	24	48
dye conc.	0.013	0.012	0.012	0.011	0.014	0.010
n	4	7	7	8	7	9

added to the sea water to act as a tracer. Comparison (t-tests) of results of volume changes in sea water with and without amaranth showed the presence of the dye did not significantly ($p=0.01$) alter the increase in volume. When limpets increased in volume in "amaranth sea water" the dye became distributed throughout the tissues. All parts of the animal "blushed" – including the gill and mantle fringe.

After volume changes, the values of amaranth in the blood were determined. There is a relationship between blood amaranth values and volume increase (Table 2). The average volume increase was around 100%, indicating the volume of water taken up was equal to the starting volume of the soft parts. The average blood dye value for these animals (0.012g/) was around ½ that of sea water (0.025g/l). It appears the increase in volume of soft parts was facilitated by bulk movement of sea water

with the dissolved dye into the fluid spaces of the limpet. The final blood dye concentration ($\frac{1}{2}$ that of sea water) would then be due to the diluting effect of the fluid of the starting volume. It is not possible to analyze more critically the relationship between water and dye uptake because (1) the intracellular distribution of amaranth, if any, was not known, and (2) the extent of the blood space at any given time was not known. Amaranth did enter the kidney space as well as the blood space. Animals stimulated to contract after volume increase in amaranth sea water would evacuate urine that was red from the dye.

Uptake of Inulin

Inulin is generally believed not to enter cells (WHITE *et al.* 1959), although SCOTT *et al.* (1964) report the absorption of inulin from the proximal tubule of *Necturus* kidney. To determine if *Acmaea scutum* could also take up inulin during volume increases limpets were placed in sea water solutions of inulin. Table 3 gives the results. Again limpets increased in volume around 100%. The average blood inulin value was 2.8g/l compared with 4g/l in the surrounding sea water. If the starting volume was considered as a water space the doubling of volume by uptake of water and 4g/l inulin would result in a final blood inulin value of 2.0g/l. However, since inulin probably did not penetrate intracellularly, the fluid available for dilution would be less and the observed blood inulin value of 2.8g/l would be expected. As with amaranth, the inulin data support the hypothesis that the increase in volume occurred by a bulk movement of sea water into the blood space.

Table 3

Blood inulin concentration (g/l) after *Acmaea scutum* had shown an increase in volume of soft body parts by taking up sea water. Each experimental salinity contained an inulin concentration of 4g/l

average % increase	n	average inulin conc.	n
113.8	30	2.85	29

Mechanism of Volume Increase

Histological sectioning showed no pores leading into the blood space. To determine if water was passing into the radula sac or intestine the volume increase was followed

in a sea water solution of the vital dye methylene blue. During the volume increase the gut but not the radula sac stained blue. To insure that it was not a case of selective staining, 1 cc of 0.1% methylene blue was injected into the blood space of each of 5 limpets. After 2 hours, the gut and radula sac were examined. Both were equally but lightly stained (2 out of a scale of 4). Moreover, when the radula and radula sac were removed from 5 limpets and stained *in vitro* in 0.1% methylene blue for 15 minutes, the radula sac was stained (3 out of a scale of 4). These data support the idea that sea water was passed into the gut. No direct evidence is available, however, that sea water passes from the gut into the blood space.

DISCUSSION

In this study it is shown that the limpet *Acmaea scutum* was capable of taking large quantities of sea water into both the blood and urine spaces. Limpets could take up a volume of sea water approximately equal to the starting volume of the soft parts. It was not possible to separately measure the extent of the increase of blood and urine spaces. Data using the vital dye methylene blue suggest that sea water passes into the blood space through the gut.

This response of *Acmaea scutum* differs from the increase in volume shown by the Naticidae (moon snails). There, the large volume increase is due to uptake of sea water into "aquiferous ducts" that are separate from the blood space (MORRIS, 1950; BERNARD, 1968; RUSSELL-HUNTER & APLEY, 1969). In *A. scutum* the sea water taken in during volume increase, in part at least, mixes directly with the blood.

It is difficult to explain how the change of volume of *Acmaea scutum* is connected to the function of the hydrostatic skeletons. Limpets cannot withdraw into their shell, so the uptake of sea water is probably not normally used for foot expansion. These experiments were performed with the limpets turned upside down. However, it is unlikely that the uptake of sea water into the fluid spaces is unique to the animal being in this position. Possibly, sea water uptake is an adaptation to intertidal exposure. The importance of free water in the mantle cavity and pallial groove (extravisceral water) of limpets in decreasing desiccation effects have been shown by SEGAL & DEHNEL (1962). When they removed the extravisceral water from *Acmaea limatula* CARPENTER, 1864, the limpet showed a more rapid increase in the total osmotic pressure of the blood when desiccated. As well, SHOTWELL (1950)

showed that animals having the greatest exposure time have the largest extravisceral water space. In *Acmaea scutum* the ability to take sea water into the circulatory system could be an important adaptation to desiccation stress in that it results in a larger fluid volume to act as an osmotic buffer.

SUMMARY

1. Evidence is presented to show that *Acmaea scutum* could increase the water content of the soft body parts at a constant salinity. The increase in water content resulted from sea water entering the fluid spaces from the external environment.
2. While taking up sea water from the external environment, the molecules inulin and amaranth could also pass into the blood space from the external sea water when these molecules were dissolved in experimental salinities.
3. Using methylene blue as a tracer, it appears that sea water enters the fluid spaces of the limpet through the gut.

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